Metabolism and Disposition of the Thienopyridine Antiplatelet Drugs Ticlopidine, Clopidogrel, and Prasugrel in Humans
Nagy A. Farid, Atsushi Kurihara and Steven A. Wrighton
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Ticlopidine, clopidogrel, and prasugrel are thienopyridine prodrugs that inhibit adenosine-5′-diphosphate (ADP)-mediated platelet aggregation in vivo. These compounds are converted to thiol-containing active metabolites through a corresponding thiolactone. The 3 compounds differ in their metabolic pathways to their active metabolites in humans. Whereas ticlopidine and clopidogrel are metabolized to their thiolactones in the liver by cytochromes P450, prasugrel proceeds to its thiolactone following hydrolysis by carboxylesterase 2 during absorption, and a portion of prasugrel’s active metabolite is also formed by intestinal CYP3A. Both ticlopidine and clopidogrel are subject to major competing metabolic pathways to inactive metabolites. Thus, varying efficiencies in the formation of active metabolites affect observed effects on the onset of action and extent of inhibition of platelet aggregation (IPA). Knowledge of the CYP-dependent formation of ticlopidine and clopidogrel thiolactones helps explain some of the observed drug-drug interactions with these molecules and, more important, the role of CYP2C19 genetic polymorphism on the pharmacokinetics of and pharmacodynamic response to clopidogrel. The lack of drug interaction potential and the absence of CYP2C19 genetic effect result in a predictable response to thienopyridine antiplatelet therapy with prasugrel. Current literature shows that greater ADP-mediated IPA is associated with significantly better clinical outcomes for patients with acute coronary syndrome.

Keywords: Clopidogrel; drug interaction; genetic polymorphism; metabolism; pharmacokinetics; platelet aggregation; prasugrel; thienopyridine; ticlopidine

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From Eli Lilly and Company, Indianapolis, Indiana (Dr Farid, Dr Wrighton) and Daiichi Sankyo Company, Limited, Tokyo, Japan (Dr Kurihara). Submitted for publication April 1, 2009; revised version accepted June 18, 2009. Address for correspondence: Nagy A. Farid, PhD, c/o Christopher S. Konkoy, PhD, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285; e-mail: nagyfarid@gmail.com; konkoycn@lilly.com.
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Platelet activation and aggregation play important roles in occlusive vascular events. Release of adenosine-5′-diphosphate (ADP) from activated platelets is one of the primary mediators of platelet aggregation, leading to a sustained response via activation of P2Y12 receptors. Inhibition of platelet aggregation with a combination of aspirin and a thienopyridine antiplatelet drug is an important strategy for preventing ischemic events in patients with acute coronary syndrome (ACS), including those undergoing percutaneous coronary intervention (PCI).’ PCI is a common procedure to treat ACS and requires periprocedural pharmacologic intervention to reduce the risk of postprocedural thrombosis. On the basis of conclusive data showing the benefit of optimal platelet inhibition on cardiovascular risk, the American Heart Association/American College of Cardiology guidelines recommend that all patients undergoing PCI receive antiplatelet therapy before and after the procedure. Because most PCI procedures employ stenting, these guidelines recommend the dual (aspirin and clopidogrel) therapy for at least 1 month after placement of a bare metal stent and for 12 months after placement of a drug-eluting stent (DES). Current European guidelines recommend that patients with non-ST segment myocardial infarction (NSTEMI) or STEMI be treated with a clopidogrel 300-mg or 600-mg loading dose (LD), followed by a daily maintenance dose (MD) of 75 mg for 12 months unless contraindicated by excessive bleeding risk.

The thienopyridines—ticlopidine, clopidogrel, and prasugrel—are oral prodrugs that require in vivo
Conversion to a thiol-containing pharmacologically active metabolite. The thiol moiety of the active metabolite binds specifically and irreversibly to cysteine residues of the platelet P2Y12 purinergic receptor, thus inhibiting ADP-mediated platelet activation and aggregation.4

Ticlopidine (Ticlid) was the first compound of this class, introduced to the market in 1979 for prevention of thrombotic stroke. The recommended ticlopidine dose is 250 mg twice daily. The most common side effects observed with ticlopidine treatment include gastrointestinal disturbances, primarily vomiting, diarrhea, nausea, dyspepsia, and purpura. In addition, skin rash appears to be a common problem.5 More serious, potentially life-threatening side effects include hemolytic anemia, nephrotic syndrome,6 hepatotoxicity,7,8 neutropenia,9 and thrombocytopenia.6,9

Clopidogrel (Plavix/Iscover) was first launched in 1998 in the United States and in European countries in 1999 for the reduction of atherosclerotic events in patients with stroke, myocardial infarction (MI), or peripheral arterial disease. The recommended daily dose was 75 mg, a dose chosen because it produced comparable inhibition of platelet aggregation to that produced by 250 mg ticlopidine twice daily.10 Adverse events noted with clopidogrel include gastrointestinal disturbances, skin rash, and purpura. Rarely, thrombotic thrombocytopenic purpura has been reported.11 Ticlopidine and clopidogrel were both shown to have significant improvements over aspirin alone in the treatment of patients with stroke or myocardial infarctions.12 In addition, patients treated with clopidogrel, as a single 300-mg LD followed by 75-mg daily MDs, and aspirin demonstrated a 20% relative risk reduction in cardiovascular events compared to the aspirin-alone group.13 However, substantial interindividual variability in the inhibition of platelet aggregation (IPA) by clopidogrel in patients has been observed, and this variability in some cases correlated with the risk of recurrent cardiovascular events. In fact, a large number of investigators observed that 15% to more than 40% of the patients responded poorly to clopidogrel. (The percentages cited depend on the methodology used to evaluate platelet aggregation and the criteria used by the investigators to determine the threshold of response.) In addition, several investigators reported a correlation between the platelet response to clopidogrel and patient outcome.14-18

Prasugrel (Effient/Efient) is the newest member of this class of drugs. In comparison to clopidogrel, recent studies demonstrated that prasugrel achieves a faster, higher, and a more consistent level of inhibition of platelet aggregation, both in healthy participants and patients with coronary artery disease.19,20 These observed differences between prasugrel and clopidogrel were evaluated to determine if they would translate into clinical differences and benefits. The TRITON-TIMI 38 study showed that prasugrel 60 mg LD/10 mg MD was superior to clopidogrel 300 mg LD/75 mg MD in preventing the composite endpoint of cardiovascular death, nonfatal MI, or nonfatal stroke in patients undergoing antiplatelet therapy for a median duration of 14.5 months.21 The benefit of prasugrel over clopidogrel was achieved with a cost of increased noncoronary artery bypass graft-related major bleeding (2.4% vs 1.8% for prasugrel and clopidogrel, respectively). The higher bleeding risk was primarily in patients less than 60 kg, patients 75 years old and older, and patients with a history of transient ischemic attack (TIA) or stroke. Net clinical benefit (rate of preventing composite endpoint vs rate of noncoronary artery bypass graft bleeding) favored prasugrel in landmark analyses of both early (up to 3 days) and late (from 3 days through the trial’s duration) events,22 consistent with the superiority of prasugrel over a standard regimen of clopidogrel for both early and ongoing pharmacologic management of patients undergoing PCI.

The PRINCIPLE-TIMI-44 study showed that prasugrel 60 mg LD produced more rapid onset, higher, and more consistent levels of platelet inhibition than clopidogrel 600 mg LD given at least 30 minutes prior to catheterization. Similarly, prasugrel 10 mg/d produced greater and more consistent levels of platelet inhibition than did clopidogrel 150 mg/d.23

Although the 3 thienopyridines share the same mechanism of action, significant differences in their metabolism, pharmacokinetics (PK), platelet response, and drug-drug interactions have been observed in studies conducted in animals and humans. Progress in understanding the reasons for these differences has evolved through elucidation of the compounds’ respective metabolic and activation pathways, potential for inhibition of cytochrome P450 (CYP) enzymes, and also the development of sensitive and specific analytical methods to determine the plasma concentrations of the active metabolites.

This review describes the metabolism, disposition, and PK of the 3 thienopyridines and how these characteristics affect platelet aggregation and clinical outcomes.
PHARMACODYNAMIC RESPONSE

Determination of the effect of thienopyridines on ex vivo platelet aggregation was the method chosen by various investigators to help evaluate their response. Studies in animals showed that the inhibition of platelet aggregation after oral dosing with the 3 thienopyridines was time and dose dependent. In these studies, it was also shown that in rats dosed with 1 of these 3 thienopyridines, prasugrel was approximately 100- and 10-fold more potent at inhibiting ex vivo platelet aggregation than ticlopidine and clopidogrel, respectively. However, in vitro, the active metabolites of clopidogrel and prasugrel were equipotent with respect to their ability to inhibit platelet aggregation, and the results provided by Yoneda et al suggested that the active metabolite of ticlopidine has approximately one tenth the potency of the other 2 thienopyridines.

BIOANALYTICAL METHODS TO DETERMINE PLASMA CONCENTRATIONS OF THE THIENOPYRIDINES AND THEIR METABOLITES

The plasma concentrations of a drug and/or its active metabolite are typically of major interest in pharmacokinetic studies. Moreover, in this class of compounds, it was eventually demonstrated that the concentrations of their respective active metabolites in human plasma were an extension of the metabolic pathways and correlated with the observed effects on platelet inhibition. For ticlopidine, methods were developed for the determination of its concentrations in human plasma. However, no methods were reported for the determination of the active metabolite of ticlopidine in plasma.

Plasma concentrations of clopidogrel were generally too low (<2 ng/mL) to be quantified after a 75-mg dose, although Nirogi et al have recently reported a sensitive liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for the determination of clopidogrel concentrations in plasma. Therefore, early bioanalytical methods quantified the pharmacologically inactive clopidogrel acid metabolite in human plasma.

Prasugrel has not been detected in human plasma, so in the initial pharmacokinetic and absorption studies with prasugrel, some of prasugrel’s inactive metabolites were measured and their derived pharmacokinetic parameters used as indicators of the absorption and metabolism of prasugrel. As reference standards for additional metabolites became available, an increased number of metabolites could be quantified. LC/MS/MS methods were developed and validated for the measurement of prasugrel’s active metabolite, R-138727, and major inactive metabolites in human plasma. The assay limit of quantitation is 0.5 ng/mL for R-138727 and 1 ng/mL for the inactive metabolites. To assist in understanding the PK and pharmacodynamics (PD) of clopidogrel through its active metabolite, an assay for the quantification of clopidogrel’s active metabolite in human plasma was also developed and validated.

To accurately determine plasma concentrations of prasugrel and clopidogrel active metabolites, these metabolites must be stabilized by derivatization with 2-bromo-3′-methoxyacetophenone immediately upon collection (in blood and prior to separation of the plasma). The omission of the derivatization step typically results in low and unreliable concentrations of the active metabolite that cannot be used in deriving meaningful conclusions with respect to PK and PK/PD relationships.

THE METABOLISM AND DISPOSITION OF TICLOPIDINE

In humans, the onset of activity after initiation of oral treatment with ticlopidine (250 mg twice daily) occurred between 24 and 48 hours, with maximal inhibition of ADP-induced platelet aggregation occurring 3 to 5 days after initial dosing. Approximately 85% of a ticlopidine oral dose was absorbed, with peak plasma concentrations (Cmax) of ticlopidine occurring 2 hours after dosing. The median ticlopidine Cmax after the first 250-mg dose was 310 ng/mL, which increased to 990 ng/mL after 21 days of dosing every 12 hours. Ticlopidine exhibited nonlinear PK, and its clearance decreased significantly after repeated dosing. The median elimination half-life (t1/2) of ticlopidine after multiple dosing was 29 hours but increased to 4 to 5 days in elderly participants. Steady-state concentrations of ticlopidine in plasma were achieved in 5 days but require 2 to 3 weeks of dosing in the elderly. These data indicate that ticlopidine inhibits its own metabolism in humans, most likely by inhibiting CYP2B6 and CYP2C19, as will be discussed later.

Ticlopidine represented 5% of the plasma radioactivity after a single dose of [14C]ticlopidine, which increased to 15% at steady state. Sixty percent of the administered radioactivity was recovered in the urine and 23% in the feces. Almost 8% of the dose was eliminated in the feces as ticlopidine, either through
excretion in the bile and/or due to the lack of absorption. Ticlopidine is 98% bound to plasma proteins.\textsuperscript{38}

Ticlopidine is rapidly metabolized by the liver. Initially, 4 ticlopidine metabolites were identified in humans, but the pharmacologically active metabolite was not detected or identified. The main metabolite, 2-chlorohippuric acid (Figure 1), appeared rapidly in the urine, whereas ticlopidine was present in the urine in only trace amounts.\textsuperscript{29,37} The N-dealkylation pathway that eventually results in 2-chlorohippuric acid formation also produced tetrahydrothienopyridine. Ticlopidine N-oxide and a hydroxy metabolite (a non-sulfur-containing compound) were also identified as metabolites (Figure 1).

Although ticlopidine was known to be a prodrug, the structure of its pharmacologically active metabolite was not known. However, the structure of ticlopidine active metabolite was deduced after the structures of prasugrel and clopidogrel active metabolites were independently disclosed in 2000.\textsuperscript{26,39} In 2004, Yoneda et al\textsuperscript{28} isolated and characterized the active metabolite of ticlopidine after in vitro incubation of 2-oxo-ticlopidine with homogenates prepared from phenobarbital-induced rat livers (Figure 1). CYP2C19 and CYP2B6 were shown to contribute to the metabolic transformation of ticlopidine to 2-oxo-ticlopidine.\textsuperscript{40,41} Dalvie and O’Connell\textsuperscript{42} demonstrated that, in vitro, CYP3A also contributes to the metabolism of ticlopidine, resulting in the formation of a pyridinium metabolite and oxidation of the carbon next to the nitrogen atom but not 2-oxo-ticlopidine. However, the CYP(s) involved in the formation of ticlopidine active metabolite from its thiolactone intermediate are unknown; likewise, CYP(s) contributing to the N-dealkylation step of ticlopidine have not been identified.

THE METABOLISM AND DISPOSITION OF CLOPIDOGREL

Clopidogrel appears to be rapidly absorbed, as determined by the appearance of its pharmacologically inactive carboxylic acid metabolite in human plasma (Figure 2). The C\textsubscript{max} of clopidogrel’s acid metabolite was 2780 ng/mL and was generally achieved 1 hour after a 75-mg clopidogrel oral dose.\textsuperscript{43} The exposure to the acid metabolite appeared proportional to clopidogrel doses between 50 and 150 mg. Clopidogrel acid metabolite accounted for 71% of the \textsuperscript{14}C concentration in human plasma at 1 hour after a 75-mg dose of [\textsuperscript{14}C]clopidogrel.\textsuperscript{44} The exposure to clopidogrel active metabolite in humans was later found to be less than dose proportional, likely due to saturable absorption, metabolism, or both.\textsuperscript{45-48} In general, quadrupling a clopidogrel dose from 75 mg to 300 mg resulted in approximately 3 times greater AUC of the active metabolite. The C\textsubscript{max} and AUC\textsubscript{0-\textsuperscript{t}} of clopidogrel active metabolite after a 300-mg LD were approximately 70 ng/mL and 90 ng·h/mL, respectively, and were approximately 30 ng/mL and 30 ng·h/ mL after a 75-mg MD. In addition, doubling the clopidogrel dose from 300 mg to 600 mg resulted in only a 44% increase in the AUC of its active metabolite. Time to C\textsubscript{max} of clopidogrel active metabolite was typically achieved between 0.5 and 1 hour after an oral dose. Clopidogrel is 98% bound to plasma proteins.\textsuperscript{59}

The effect of daily 75-mg doses of clopidogrel on platelet aggregation typically reaches steady state
3 to 7 days after initiation of treatment. Cadroy et al showed that during the first 24 hours of a 75-mg clopidogrel dose given with aspirin, IPA was not significantly different from predose values. However, a single 300-mg clopidogrel LD resulted in platelet inhibition values 6 hours after dosing that were comparable to those achieved after a few days of dosing with 75 mg clopidogrel. This highlighted the importance of administering an LD to patients with ACS, with or without coronary stent implantation, in which effective antithrombotic responses are needed as early as possible to prevent thrombosis. Thebault et al reported that single loading doses of clopidogrel 100, 200, 400, and 600 mg dose dependently increased ex vivo IPA induced by 5 μM ADP up to the 400-mg dose; no further increase in IPA was observed for the 600-mg dose. Several other investigators evaluated the IPA of clopidogrel 600 mg and 900 mg against the 300-mg LD. In most cases, the 600-mg LD increased mean IPA between 10 and 20 percentage points, 4 to 6 hours after dosing, compared with the 300-mg LD. However, the 900-mg LD did not provide any significant additional increase in IPA. These observations support the concept that increases in clopidogrel dosing produce less than proportional increases in clopidogrel active metabolite concentrations. An important finding in all of these reports was that intersubject variability in the response to the clopidogrel 300-mg LD did not decrease by increasing the dose to 600 mg.

The first clopidogrel metabolite identified was the carboxylic acid derivative (Figure 2), which was later shown to be produced through hydrolysis by human carboxylesterase 1 (hCE1), primarily a hepatic carboxylesterase. In initial studies investigating the in vivo formation of clopidogrel active metabolite, Savi et al demonstrated the importance of hepatic metabolism in eliciting clopidogrel’s effect on platelet aggregation and that the active metabolite was formed through an intermediate, 2-oxo-clopidogrel (Figure 2). Kazui et al demonstrated that clopidogrel remained essentially unchanged when incubated with a human small intestine S9 fraction but was hydrolyzed to its carboxylic acid metabolite when incubated with a human liver S9 fraction in the absence of NADPH and to the carboxylic acid and 2-oxo-clopidogrel in the presence of NADPH. Incubation of clopidogrel or 2-oxo-clopidogrel with human liver microsomes and NADPH, in the presence of a reducing agent (such as glutathione), produced the clopidogrel active metabolite (Figure 2).

The absolute S-configuration at the benzylic carbon of clopidogrel, which was found to be stable in vivo, is required for biological effect on platelets, and so is the Z configuration of the ethylenic bond at carbon 3. However, the effect of the configuration of the thiol group of the active metabolite on the expression of activity on platelets was not determined.

The CYP forms involved in producing the pharmacologically active metabolite from clopidogrel were identified as CYP1A2 in rats and CYP3A in humans. In addition, several recent clinical studies have shown that loss of catalytic activity of CYP3A4, CYP3A5, or CYP2C19 due to enzyme inhibition or genetic polymorphism has a negative effect on the PK of clopidogrel active metabolite and its effect on platelets, indicating that these CYPs play a significant role in the metabolism of clopidogrel to its active
METABOLISM AND DISPOSITION OF THIENOPYRIDINES

Figure 3. Prasugrel metabolic pathway of interest.

The contribution of human CYP1A2 to the formation of the thiolactone, 2-oxo-clopidogrel, has been confirmed by Kurihara et al. These authors also demonstrated that CYP2C19 is a major contributor to 2-oxo-clopidogrel formation from clopidogrel, followed by CYP2B6. Furthermore, by following the formation of the product of each oxidative step by LC/MS/MS, these authors clearly demonstrated that CYP3A does not contribute to the formation of the thiolactone metabolite from clopidogrel; rather, CYP3A contributes to the formation of the active metabolite from the thiolactone. In total, 4 CYP forms contribute to the formation of clopidogrel active metabolite from 2-oxo-clopidogrel: CYP3A, CYP2B6, CYP2C19, and CYP2C9.

The above indicates that 2 competing metabolic pathways occur in the liver: the hydrolysis of clopidogrel by hCE1 to form its inactive acid metabolite and the CYP-catalyzed oxidation of clopidogrel to 2-oxo-clopidogrel. Similarly, there are 2 competing pathways for 2-oxo-clopidogrel: its hydrolysis by hCE1 to its carboxylic acid analog and its oxidation by CYP3A, CYP2B6, CYP2C19, and CYP2C9 to the active metabolite. Indeed, Hagihara et al recently reported that following incubation of clopidogrel in human liver microsomes, about 90% of clopidogrel was converted to its carboxylic acid metabolite, that 2-oxo-clopidogrel accounted for 224 fmol/min/µM of clopidogrel in the incubation mixture, and that approximately 50% of the produced thiolactone was hydrolyzed to the corresponding inactive acid. In total, these data suggest that only a small percentage (ie, 10% or less) of a clopidogrel dose is ultimately converted to its active metabolite.

THE METABOLISM AND DISPOSITION OF PRASUGREL

Studies have shown that prasugrel is rapidly absorbed, is extensively metabolized (Figure 3), and is not detected in human or animal plasma. The Cmax of its pharmacologically active metabolite, R-138727, was achieved about 30 minutes after an oral dose. The exposure to prasugrel metabolites is essentially proportional to the administered dose in humans. Following a 60-mg LD and a 10-mg MD, the Cmax values for R-138727 were typically about 453 ng/mL and 56 ng/mL, respectively, and the corresponding AU0-30 values were 460 and 54 ng h/mL. R-138727 was detectable in human plasma for about 24 hours after a 60-mg LD and for 6 to 8 hours after a 10-mg MD (data on file, Daiichi Sankyo, Inc. and Eli Lilly and Company). The concentrations of R-95913 and R-119251 peaked at 0.5 hours, the same time as R-138727, and declined in parallel with each other and with the active metabolite. Concentrations
of R-106583 were higher than those of R-138727, peaked at 1 hour, and declined slower than those of R-138727 and the 2 other metabolites. These metabolites did not accumulate during multiple dosing. Approximately 21% of a 15-mg [14C]prasugrel dose lites did not accumulate during multiple dosing. 

R-138727 and the 2 other metabolites. These metabo-

peaked at 1 hour, and declined slower than those of R-106583, which were higher than those of R-138727, 

of R-106583 were higher than those of R-138727, 

of prasugrel was evaluated for their effects on IPA in stable aspirin-treated patients with coronary artery disease. In this population, a 40-mg or 60-mg LD of prasugrel produced a faster onset of IPA compared with 300 mg clopidogrel and also a higher degree of inhibition: 70% for the 60-mg prasugrel dose compared with 31% for the 300-mg clopidogrel dose, 6 hours after the dose. A dose-dependent effect of the daily MD of prasugrel on the IPA was observed, with the 5-mg prasugrel dose resulting in essentially similar IPA to the 75-mg clopidogrel dose, 34.5% versus 31.2%, respectively, whereas the IPA after 10-mg daily doses of prasugrel was 57.5%. This and additional clinical studies led to the selection of prasugrel loading and maintenance doses of 60 mg and 10 mg, respectively, for subsequent clinical evaluations.

The antiplatelet effects of prasugrel 60 mg LD and 10 mg MD were compared in several studies to the responses of clopidogrel 300- and/or 600-mg LDs and 75- or 150-mg MDs. Typically, prasugrel 60 mg LD achieved IPA values (20 μM ADP) of about 50% thirty minutes after the dose, which increased to approximately 75% to 80% at 1 hour after the dose. In these studies, maximum IPA with clopidogrel 300 mg and 600 mg was 50% and 69%, respectively, and occurred 6 hours after the dose. In addition, a 10-mg MD of prasugrel produced mean IPA values that were consistently higher than those obtained by either 75 mg or 150 mg of clopidogrel. Another finding of these studies was that prasugrel produced more consistent IPA between participants compared with clopidogrel.

Prasugrel, like ticlopidine and clopidogrel, is extensively metabolized and is now the best characterized of the thienopyridines with respect to metabolism. The hydrolysis of prasugrel, which is mediated by hCE2, primarily an intestinal enzyme, forms the thiolactone R-95913 (Figure 3) through keto-enol tautomerism. R-95913 is metabolized to prasugrel’s active metabolite, R-138727, which is then metabolized to 2 inactive compounds: R-106583 (by S-methylation) and R-119251 (by conjugation with cysteine). (Figure 3). R-119251 contains a disulfide bond, which can be reduced in vivo back to R-138727.

After a single 15-mg [14C]prasugrel dose to human participants, R-106583 was the major metabolite circulating in plasma, representing 26% of the AUC0-12 h of the radioactivity. The 4 key metabolites—R-95913, R-138727, R-119251, and R-106583—combined comprised approximately 70% of the plasma radioactivity at 15 minutes and 30 minutes after dosing, suggesting rapid metabolism of prasugrel via the pathway leading to the active metabolite (data on file, Study H7T-LC-TAAB, Daiichi Sankyo, Inc. and Eli Lilly and Company). The AUC0-0.25 h of R-138727 and its 2 downstream metabolites, R-106583 and R-119251, accounted for about 55% of the comparable area for plasma radioactivity (Table I). These data suggest strongly that at least half of the prasugrel dose was rapidly converted to its active metabolite. This is further supported by the elimination results of prasugrel metabolites in human urine and feces in which metabolites not derived from the active metabolite pathway were estimated to account for roughly 53% of the administered dose (data on file, Study H7T-LC-TAAB, Daiichi Sankyo, Inc. and Eli Lilly and Company).

The single major metabolite of prasugrel found in human urine was M1 (Figure 3), which accounted for 21.3% ± 5.1% (mean ± SD; n = 5) of the dose. The major metabolites found in feces were R-106583 and M1. The formation of M1 from prasugrel was proposed to be through a pathway that involves an isomer of R-95913, which was metabolized to a thione. Formation of the corresponding ketone from the thione, followed by reduction, yielded M1. It is likely that formation of the hydroxy metabolite of ticlopidine (Figure 1) follows a similar pathway.

Both hCE1 and hCE2 are capable of catalyzing the conversion of prasugrel to R-95913, but the hydrolysis rate with hCE2 was 25 times that with hCE1. It was also found that incubation of prasugrel in human small intestine S9 fraction resulted in a rapid decrease in prasugrel concentration and a corresponding increase in R-95913 formation. In addition,
results of a Caco-2 monolayer metabolism and transport study demonstrated the complete conversion of prasugrel to R-95913 during absorption. Regardless of apical or basolateral administration of prasugrel to the Caco-2 cells, prasugrel was found only in the donor buffer and not the receiver buffer, strongly suggesting that prasugrel does not pass through the intestine intact.71 Thus, it was concluded that all of R-95913 was formed during the absorption of prasugrel through the intestine. The extent of R-95913 formation from prasugrel was later determined in vitro following incubation of prasugrel in human liver microsomes (which contain hCE1). R-95913 was produced at a rate of 5520 fmol/min/µM of prasugrel in the incubation mixture, even without the contribution of hCE2.65 Four CYPs are capable of forming prasugrel active metabolite R-138727 from its thiolactone, R-95913. The main contributors are CYP3A4 and CYP2B6, with smaller contributions by CYP2C9 and CYP2C19.72 Additional in vitro data showed that CYP3A4 and CYP3A5 are similarly efficient in converting R-95913 to R-138727.73 Prasugrel was developed as a racemate because the administration of either of its enantiomers to dogs resulted in the equal formation of the 4 stereoisomers of R-95913. Thus, R- and S-configurations at the benzylic carbon of prasugrel interconvert in vivo.74 Like R-95913, R-138727 contains 2 chiral centers, and its 4 stereoisomers have varying affinities for the P2Y12 receptor, with the (R,S) and (R,R) isomers being the most potent (the first letter refers to the configuration of the carbon carrying the thiol group) (Figure 4).75 In humans, the (R,S) and (R,R) isomers (R-125690 and R-125689, respectively) comprised about 84% of the R-138727 in plasma, with the (S,R) and (S,S) pair (R-125688 and R-125687, respectively) accounting for about 16%. The ratio was consistent among participants, regardless of the dose, time of sample collection, or whether the blood was sampled after the first dose of the drug or after a few weeks of pharmacotherapy with prasugrel.76 This stereoselectivity in the disposition of R-138727 could be due to its stereoselective formation, metabolic clearance, or a combination of both. The observed stereoselectivity in the active metabolite formation in humans from R-95913 suggests that either the opening of the thiolactone ring in R-95913, a CYP-mediated process,72 is stereoselective with chirality preserved up to the step leading to R-138727 formation, or that the ultimate step leading to R-138727 formation is stereoselective, as described previously.76 Two in vitro studies may support this stereoselectivity in the disposition of R-138727. In the first study, the ratio of the R-138727 isomer pairs (calculated as [(R,S) + (R,R)]/[(S,R) + (S,S)]) after incubating the expressed CYP3A4 or CYP3A5 with 20 µM R-95913 was 1.6.73 In the second study, the S-methylation of R-138727 by thiol S-methyl transferase appeared to be stereoselective, favoring the formation of the S-methyl analogs of the least potent isomer pair (S,R and S,S) of R-138727.77 Together with the stereoselective formation of the metabolites by the CYP3A forms, this finding may partly explain the in vivo profile of R-138727 in plasma where the RS- and RR-forms are the major enantiomers.

Table I Exposure to Prasugrel Active and Inactive Metabolites as a Percentage of the Plasma Radioactivity in Humans After a Single 15-mg Oral Dose of [14C]Prasugrel

<table>
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<th>Metabolite</th>
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<th>Percentage of 14C</th>
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<td></td>
<td>Concentration</td>
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</table>

Data on file, Study H77-LC-TAAAB, Daiichi Sankyo, Inc and Eli Lilly and Company.
after a prasugrel 60-mg LD, but the overall exposure (AUC) was bioequivalent with and without ketoconazole. This change in R-138727 C\textsubscript{max} was consistent with the effect of inhibition of intestinal CYP3A activity by ketoconazole on the initial formation of R-138727 during the absorption process. In the same study, the AUC of R-95913 doubled, whereas its C\textsubscript{max} increased by 71% to 93%, with no change to t\textsubscript{max} or t\textsubscript{1/2} (Table II). Insofar as AUC and C\textsubscript{max} reflect bioavailability whereas t\textsubscript{1/2} depends directly on hepatic clearance, these differential pharmacokinetic effects on R-95913 AUC and C\textsubscript{max} are indicative of a substrate for which metabolism by intestinal CYP3A is important, but hepatic CYP3A-mediated metabolism is less important. These results also suggest that R-95913 has secondary metabolic pathway(s) that may be CYP3A dependent, in addition to that resulting in R-138727 formation. This is supported by the observation that coadministration of the CYP inducer rifampin with prasugrel resulted in reduction in the C\textsubscript{max} and AUC of R-95913 by 68% to 84%, compared to prasugrel alone, while not affecting the exposure to the active metabolite or the 2 downstream metabolites (Table II).

Significant involvement of intestinal enzymes also helps explain the rapid appearance of prasugrel’s active metabolite in plasma and the lack of effect of moderate hepatic impairment on the PK of prasugrel’s active metabolite. Based on both the in vitro and in vivo data, it was concluded that a significant portion of R-95913 is oxidized to the active metabolite of prasugrel during intestinal absorption. 

A comparison of the key PK and PD data for ticlopidine, clopidogrel, and prasugrel is presented in Table III.

**EFFECT OF THE THIENOPYRIDINES ON CYP CATALYTIC ACTIVITY**

In vitro, ticlopidine was found to be a potent mechanism-based inhibitor of CYP2B6 and CYP-2C19\textsuperscript{38,81} and is also an inhibitor of CYP1A2 and CYP2D6.\textsuperscript{81-83} In addition, 2-oxo-ticlopidine inhibited CYP2B6 with similar potency to ticlopidine itself.\textsuperscript{84} Clopidogrel was found to be a mechanism-based inhibitor of CYP2B6\textsuperscript{84} and an inhibitor of CYP2C19 (IC\textsubscript{50} = 0.524 µM).\textsuperscript{83,85} However, 2-oxo-clopidogrel was a much weaker inhibitor of CYP2B6 and CYP2C19, such that its ability to inhibit these 2 isoforms would not be of clinical importance.\textsuperscript{38,84} The thiol-containing active metabolites of ticlopidine and clopidogrel, as well as the major circulating metabolite of clopidogrel, the acid derivative, were not found to be inhibitors of the various CYP forms (IC\textsubscript{50} > 50 µM).\textsuperscript{81}

Because prasugrel is rapidly hydrolyzed in the intestine and is not detected in plasma, in vitro CYP inhibition studies were conducted with the hydrolysis product, the thiolactone R-95913. In vitro, R-95913 inhibited CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, with K\textsubscript{i} values ranging from 7.2 µM to 82 µM, but did not inhibit CYP1A2.\textsuperscript{72} These K\textsubscript{i} values significantly exceeded the highest observed C\textsubscript{max} of R-95913 (248 nM) in any participant receiving a 10-mg prasugrel daily dose (data on file, Study
METABOLISM AND DISPOSITION OF THIENOPYRIDINES

H7T-LC-TAAV, Daiichi Sankyo, Inc. and Eli Lilly and Company). In addition, prasugrel active metabo-
lite, R-138727, and its main circulating metabolite,
R-106583, did not inhibit any of the CYPs indi-
cated above.72 Thus, prasugrel’s metabolites R-95913,
R-138727, and R-106583 are unlikely to inhibit the
CYP-mediated metabolism of coadministered drugs.

Clinical studies confirmed several of the in vitro
findings regarding the abilities of the thienopyridines
to inhibit various CYP forms. Coadministration of
ticlopidine with theophylline to healthy participants
decreased theophylline clearance from 0.682 mL/kg/
min to 0.431 mL/kg/min and increased its elimina-
tion t1/2 from 8.6 to 12.2 hours.86 Results of this
study suggested that ticlopidine inhibits CYP1A2 in
humans.87 Clopidogrel did not affect the PK of theo-
phylline.87 The ability of ticlopidine, but not clopi-
dogrel, to inhibit the metabolism of compounds
metabolized by CYP1A2 may be explained by the
differences in their plasma concentration. Although
the IC50 values for ticlopidine and clopidogrel for
the inhibition of CYP1A2 (12.4 µM and 24.3 µM, respec-
tively) are of comparable magnitude,83 the Cmax values

of ticlopidine at steady state (during 250-mg twice-
daily dosing) are in the 3-µM to 8-µM range, whereas
those for clopidogrel are typically <14 nM.

Symptomatic phenytoin toxicity has been reported in
patients administered both ticlopidine and phenytoin,
suggesting that ticlopidine inhibits CYP2C19 in
humans.88,89 Subsequently, ticlopidine was found
to decrease the activity of CYP2C19 in humans,
resulting in a significant decrease in apparent
clearance of omeprazole from 25.7 L/h to 10.8 L/h.90
Leiri et al91 reported that although the PK of omepra-
Zole were different between participants who are
CYP2C19 homozygous extensive metabolizers, CYP
2C19 heterozygous extensive metabolizers, and
CYP2C19 homozygous poor metabolizers, these dif-
fferences disappeared after coadministration of
ticlopidine (200 mg/d), with all participants dem-
onstrating the pharmacokinetic profile of the homozy-
gous poor metabolizers and indicating the inhibition
of CYP2C19 by ticlopidine.

The observed in vitro inhibitory effect of ticlopi-
dine and clopidogrel on CYP2B6 was confirmed
in a clinical study using bupropion as a substrate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prasugrel Alone (n = 18)</th>
<th>Prasugrel Alone (n = 30)</th>
<th>Prasugrel + Ketoconazole (n = 18)</th>
<th>Prasugrel + Ketoconazole (n = 29)</th>
<th>Prasugrel + Rifampin (n = 29)</th>
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<tbody>
<tr>
<td></td>
<td>Geometric Mean (CV%)</td>
<td>Geometric Mean (CV%)</td>
<td>Geometric Mean (CV%)</td>
<td>Geometric Mean (CV%)</td>
<td>Geometric Mean (CV%)</td>
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<tr>
<td>Prasugrel 60-mg LD</td>
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<td></td>
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<td></td>
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<tr>
<td>AUC0-t, ng h/mL</td>
<td>332 (45.8)</td>
<td>371 (32.7)</td>
<td>660 (35.6)</td>
<td>98.6 (31.6)</td>
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<tr>
<td>Cmax, ng/mL</td>
<td>193 (56.3)</td>
<td>172 (40.1)</td>
<td>331 (37.3)</td>
<td>54.7 (43.0)</td>
<td>54.7 (43.0)</td>
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</tr>
<tr>
<td>AUC0-t, ng h/mL</td>
<td>90.9 (50.9)</td>
<td>58.6 (41.6)</td>
<td>191 (33.2)</td>
<td>9.31 (48.8)</td>
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<tr>
<td>Cmax, ng/mL</td>
<td>52.6 (41.4)</td>
<td>33.1 (38.3)</td>
<td>102 (38.4)</td>
<td>6.98 (51.2)</td>
<td>6.98 (51.2)</td>
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</table>

Table II  Effect of Enzyme Inhibition and Induction on the Exposure Parameters for the Thiolactone of Prasugrel, R-95913, in Healthy Participants When Prasugrel Is Administered Alone and With Ketoconazole or Rifampin

Data on file, Study H7T-EW-TAAK, Daiichi Sankyo, Inc. and Eli Lilly and Company and Farid et al.79 CI, confidence interval; CV, coefficient of variation; LD, loading dose; LS, least squares; MD, maintenance dose.
because its hydroxylation is a pathway almost exclusively catalyzed by CYP2B6.92-94 Ticlopidine and clopidogrel increased the mean AUC for bupropion by 85% and 60%, respectively, and decreased the mean AUC of hydroxybupropion by 84% and 52%.94 Prasugrel was found to have a weaker inhibitory effect on CYP2B6 in humans because the AUC of hydroxybupropion was 9.64 µg⋅h/mL when bupropion was given with prasugrel versus 12.3 µg⋅h/mL when given alone, a decrease of 23%.95 There have been no other reports to indicate inhibitory effects of prasugrel or clopidogrel on other CYP forms in humans.

Taken together, these data suggest that the mechanism-based inhibition of the catalytic activity of CYP2B6 and CYP2C19 by ticlopidine and clopidogrel occurs during the first oxidative step that leads to the formation of their respective thiolactones. Because the formation of prasugrel’s thiolactone, R-95913, is not CYP dependent, such an inhibitory effect would not occur during R-95913 formation.

**EFFECT OF CYP INHIBITION OR GENETIC POLYMORPHISMS ON THE THIENOPYRIDINES**

This area of investigation has gained significant momentum over the past 6 years because of observations regarding the effect of CYP inhibition or genetic polymorphism on the platelet response to clopidogrel therapy. One of the main contributing factors to this area of interest was the identification of patients who respond poorly to clopidogrel, as previously mentioned.13-18,70 In 2003, Lau et al96 reported that atorvastatin, a CYP3A substrate, but not pravastatin, which is metabolized by conjugation, reduced the inhibitory effect of clopidogrel on platelet aggregation in a dose-dependent manner. However, the number of participants per atorvastatin dose group was very small. Since the initial study, a few reports have agreed with that finding, whereas several others have not.97 A prospectively designed, statistically powered crossover study was conducted to assess the effect of atorvastatin, given at the highest approved dose, 80 mg, on the platelet response to clopidogrel (300 mg LD/75 mg MD) or prasugrel (60 mg LD/10 mg MD). The pharmacokinetic parameters for each active metabolite were determined as well as the extent of IPA when each thienopyridine was given alone and with atorvastatin. The results showed that statin administration did not negatively affect the PK or PD response to either prasugrel or clopidogrel.46

Because CYP3A forms contribute to the formation of the active metabolites of clopidogrel and prasugrel from their respective thiolactones, the effect of CYP3A4 and CYP3A5 inhibition with ketoconazole on the PK of both active metabolites and their effects on platelets were determined in healthy participants in a randomized crossover study.45 The participants received an LD/MD of prasugrel 60/15 mg or clopidogrel 300/75 mg (5 daily MDs) without or with ketoconazole at 400 mg/d. Ketoconazole decreased the Cmax values of the active metabolites 34% to 61% after prasugrel and clopidogrel dosing. Although ketoconazole did not affect R-138727 AUC or prasugrel’s inhibition of platelet aggregation, it decreased clopidogrel’s active metabolite AUC0-24 22% (LD) to 29% (MD) and reduced inhibition of platelet aggregation 28 percentage points (LD) to 33 percentage points (MD). This study showed that CYP3A4 and

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**Table III**  Summary of Thienopyridine Pharmacokinetics and Pharmacodynamics

<table>
<thead>
<tr>
<th></th>
<th>Ticlopidine, 250 mg bid&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Clopidogrel, 300/75 mg (LD/MD)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Prasugrel, 60/10 mg (LD/MD)&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>Pharmacokinetic estimates</td>
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<td></td>
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</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt;, h</td>
<td>2.0&lt;sup&gt;28&lt;/sup&gt;</td>
<td>0.5-1.0&lt;sup&gt;47&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;47&lt;/sup&gt;</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, ng/mL</td>
<td>990&lt;sup&gt;28&lt;/sup&gt;</td>
<td>70/28&lt;sup&gt;46&lt;/sup&gt;</td>
<td>453/56&lt;sup&gt;46&lt;/sup&gt;</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t&lt;/sub&gt;, ng⋅h/mL</td>
<td>4060&lt;sup&gt;28&lt;/sup&gt;</td>
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</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt;, h</td>
<td>29&lt;sup&gt;28&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.4</td>
</tr>
<tr>
<td>Pharmacodynamic response (20 µM ADP)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPA (time from LD), % (h)</td>
<td>NA</td>
<td>43 (6)&lt;sup&gt;46&lt;/sup&gt;</td>
<td>79 (1)&lt;sup&gt;46&lt;/sup&gt;</td>
</tr>
<tr>
<td>IPA (MD), %</td>
<td>20-30&lt;sup&gt;109,110&lt;/sup&gt;</td>
<td>34-52&lt;sup&gt;46&lt;/sup&gt;</td>
<td>60-71&lt;sup&gt;46&lt;/sup&gt;</td>
</tr>
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</table>

ADP, adenosine-5′-diphosphate; IPA, inhibition of platelet aggregation; LD, loading dose; MD, maintenance dose.

<sup>a</sup>Steady-state pharmacokinetic parameters reported for ticlopidine.

<sup>b</sup>Respective active metabolite pharmacokinetic parameters reported for clopidogrel and prasugrel.

<sup>c</sup>Clopidogrel active metabolite concentrations are typically below the assay limit of quantitation (0.5 ng/mL) by 2 to 4 hours after the dose (eg, see Farid et al<sup>46</sup>), making reliable estimation of its terminal t<sub>1/2</sub> unattainable.
CYP3A5 inhibition by ketoconazole affects the extent of active metabolite formation of clopidogrel, but not prasugrel, and that the decreased formation of clopidogrel active metabolite was associated with its reduced ability to inhibit platelet aggregation. Another study by Suh et al. showed that administration of itraconazole, a selective CYP3A4 inhibitor, with clopidogrel to participants who did not express CYP3A5 lacked the ability to inhibit platelet aggregation. They also reported that atherothrombotic events tended to occur more frequently in patients treated with clopidogrel who did not express CYP3A5.

Another enzyme that contributes to both oxidative steps of clopidogrel metabolism is CYP2C19, a polymorphic enzyme. Two of the earliest publications to describe the effect of CYP2C19 polymorphism on clopidogrel were by Hulot et al. and Brandt et al. Hulot et al showed that participants who were CYP2C19 extensive metabolizer (EM) homozygotes (*1/*1) had the ability to inhibit platelet aggregation after daily 75 mg clopidogrel over a 7-day period. Maximum platelet aggregation (MPA) in response to 10 μM ADP decreased from 76.2% ± 6.8% predose to 48.9% ± 14.9% on day 7 of treatment (P < .001). However, there was no effect of clopidogrel on platelet aggregation in the CYP2C19 heterozygous (EM/poor metabolizer [PM]) participants (*1/*2; baseline was 77.2% ± 4.3% vs 71.8% ± 14.6% on day 7, P = .22), suggesting that the formation of the clopidogrel active metabolite was significantly reduced in the heterozygous population. In the second study, the PK of and the PD response to a clopidogrel 300-mg LD and a prasugrel 60-mg LD were evaluated in a retrospective analysis of 2 clinical trials that included 71 participants. The authors found that genetic variations in CYP2C19 resulting in the loss of catalytic function were associated with decreased exposure to the active metabolite of clopidogrel and thus decreased its ability to inhibit platelet aggregation. There was no effect of CYP2C19 polymorphisms on the PK of prasugrel active metabolite or, accordingly, its effect on platelets. Recently, Kim et al. demonstrated that exposure to clopidogrel prodrug correlated with CYP2C19 genotype after treating participants with a 300-mg clopidogrel LD, followed by a 75-mg dose for 2 days. Exposure to clopidogrel was lowest in the homozygous EMs (CYP2C19*1/*1), intermediate in the heterozygous (EM/PM) group (*1/*2 and *1/*3), and highest in the PMs (*2/*2 and *2/*3). Accordingly, the ability of clopidogrel to inhibit platelet aggregation decreased in that same order. Studies in patients undergoing PCI also demonstrated that the presence of a CYP2C19*2 allele was associated with decreased clopidogrel-inhibited platelet aggregation and may be associated with poorer clinical outcome compared with patients who do not carry this loss-of-function allele. Similarly, Close et al. reported that of 349 healthy participants, those who were carriers of the reduced-function CYP2C19 allele had a 53% (P = .0003) reduction in plasma AUC of the clopidogrel active metabolite after a 300-mg LD, resulting in less reduction (by 19 percentage points) in MPA, compared with those classified as EMs, 4 hours after clopidogrel 300 mg LD (P = .04). The CYP2C19 genetic effect on response to clopidogrel was consistently observed after both the LD (600 and 300 mg) and MD (75 mg). These observations have been substantiated in larger studies that concluded that among ACS patients treated with clopidogrel, carriers of a CYP2C19 reduced-function allele had diminished platelet inhibition, resulting in higher rates of stent thrombosis and major adverse cardiovascular events. For prasugrel, none of the genetic variants tested had a clinically or statistically significant effect on active metabolite exposure or reduction in P2Y₁₂ inhibition.

Small et al. assessed the effect of the proton pump inhibitor (PPI) lansoprazole on the PK and PD of loading doses of prasugrel (60 mg) and clopidogrel (300 mg) in healthy participants. Although lansoprazole coadministration resulted in a 19% decrease in prasugrel active metabolite AUC, there was no effect on the IPA after the prasugrel dose. Lansoprazole did not affect the exposure to clopidogrel inactive acid metabolite but resulted in lower IPA after the clopidogrel dose, suggesting an effect on the extent of clopidogrel active metabolite formation. (The active metabolite was not measured because its assay had not been developed at the time of study.) Retrospective tertile analysis showed that the negative effect of lansoprazole on IPA after dosing with clopidogrel was most evident in the participants who responded best to clopidogrel alone. The analysis also showed that although lansoprazole lowered IPA in participants treated with clopidogrel, it had no effect on the IPA response to prasugrel in these same participants.

The clearance of both PPIs omeprazole and esomeprazole (the S-enantiomer of omeprazole, a CYP2C19 substrate and inhibitor) decreases with repeated dosing, suggesting that these compounds impair the enzymatic activity of CYP2C19. Gilard et al. reported that omeprazole significantly reduced the ability of clopidogrel to inhibit platelet aggregation in patients undergoing coronary stent implantation.
In contrast, Siller-Matula et al.\textsuperscript{107} found that coadministration of either esomeprazole or pantoprazole with clopidogrel did not affect the platelet response to clopidogrel, implying the absence of a class effect among PPIs. Lack of information regarding identification of patients as responders or nonresponders to clopidogrel before initiation of treatment may underlie the apparent lack of agreement between the results of these studies. Considering the significant role CYP2C19 plays in the bioactivation of clopidogrel, ideally a prospectively designed, statistically powered crossover design study in clopidogrel responders is needed before a conclusion can be made regarding possible interaction between clopidogrel and omeprazole or esomeprazole. Moreover, based on the known differences in the hepatic metabolism of the PPIs, and because other PPIs have not been shown to be CYP2C19 inhibitors in vivo, these observations will most likely be limited to omeprazole and esomeprazole.

DISCUSSION

Antiplatelet therapy has proven to be crucial for managing patients with ACS and coronary artery disease and in patients undergoing PCI. Clopidogrel differs from ticlopidine by the addition of the carboxymethyl group at the benzylic carbon. The addition of this side chain apparently decreases or possibly eliminates the metabolic pathway that leads to the cleavage of ticlopidine into the thienopyridine and $\alpha$-chlorobenzyl moieties. It also appears that the increased lipophilic character in the benzylic region may result in improved potency toward the P2Y\textsubscript{12} receptor, thus increasing IPA, because the active metabolite of clopidogrel is approximately 10 times more potent than that of ticlopidine, as mentioned above. The combination of these 2 factors would explain why the use of a lower daily clopidogrel dose, 75 mg, produces the same degree of platelet inhibition in humans as that produced by 500 mg of ticlopidine daily. Both ticlopidine and clopidogrel are metabolized in the liver. One could easily predict the involvement of CYP forms in the formation of ticlopidine and clopidogrel thiolactones in the first of 2 steps in their biotransformation to their respective active metabolites because 1 oxygen atom is introduced into each compound at the second position of the thiophene ring. However, in vitro data showed that CYP3A does not contribute to the formation of the thiolactones of ticlopidine and clopidogrel.\textsuperscript{52,64}

Structurally, clopidogrel and prasugrel differ in several key elements. Prasugrel contains a cyclopropylcarbonyl group at the benzylic carbon atom compared with the carboxymethyl group at this position in clopidogrel, thus imparting stability toward enzymatic hydrolysis at this site. Another key difference is the presence of the acetoxyl group at the second position of the thiophene ring in prasugrel, thus eliminating the need of CYP-dependent oxidation to produce the thiolactone. The net effect is that upon complete hydrolysis by carboxylesterases, the formed thiolactone is readily available for the CYP-dependent step that produces the active metabolite. Replacement of the chlorine atom of clopidogrel by fluorine in prasugrel also appears to improve the antiplatelet effect in the rat (US Patent 5,288,726 [1994]).

The net effect of differences between the metabolic pathways of prasugrel and clopidogrel is that significantly more of the prasugrel thiolactone intermediate is formed relative to that for clopidogrel. This results in the formation of more active metabolite after a prasugrel dose compared with a clopidogrel dose. The data presented here show that the thiolactone and the active metabolite of prasugrel are formed faster during the absorption/first-pass process compared with clopidogrel. As stated above, a 10-mg dose of prasugrel produces twice as much of its active metabolite compared to the 75-mg clopidogrel dose, and a 60-mg dose of prasugrel produces 5 times the active metabolite produced from a 300-mg LD of clopidogrel (calculated from AUC data). Clinically, this translates into having a faster onset of action, greater extent of IPA, and a more consistent response to treatment for participants on prasugrel relative to those on clopidogrel. In addition, because the formation of prasugrel’s thiolactone is not CYP dependent, the potential for clinically significant drug interactions between other CYP-metabolized drugs and prasugrel is essentially eliminated. In addition, there is less chance for other enzyme inhibitors or genetic polymorphisms to alter the pharmacodynamic effect of prasugrel on platelets.

Knowing that the pharmacologically active metabolites of prasugrel and clopidogrel are equipotent, the ability to quantify these metabolites in human plasma enabled the development of a correlation (Figure 5) between exposure to the active metabolite and the extent of IPA.\textsuperscript{108} The correlation showed that the active metabolites of prasugrel and clopidogrel have the same exposure-IPA relationship and aided the interpretation of clinical observations and findings. Thus, the platelet-inhibitory effects of the active metabolites of prasugrel and clopidogrel depend on their respective AUCs. Whereas a 60-mg prasugrel LD results in a pharmacodynamic response at or near the $E_{\text{max}}$, that from a 300-mg clopidogrel LD will fall.
on the relatively steep portion of the dose-response curve, near and within the ascending part of the exposure-pharmacodynamic curve and below the E\text{max}. This suggests that relatively small changes in exposure to active metabolite alter the pharmacodynamic response to clopidogrel while having essentially no effect on prasugrel (Figure 5).

CONCLUSION

The thienopyridines ticlopidine, clopidogrel, and prasugrel are prodrugs that require biotransformation to produce their respective active metabolites. There are significant differences in the metabolic pathways leading to the formation of each compound’s active metabolite. These metabolic differences result in varying efficiencies in the production of active metabolite and hence in the observed effects on the onset of action and extent of IPA. In addition, differences in the metabolic pathways, particularly the pathways leading to thiolactone intermediate formation, have proven to be critical in differentiating between the molecules with respect to (1) their potential for drug-drug interactions and (2) the impact of genetic polymorphisms on PK, IPA, and clinical outcomes. For prasugrel, the lack of drug interaction potential and absence of effect of genetic polymorphisms should result in more predictable clinical responses. Although the target levels of ex vivo inhibition of platelet aggregation induced by ADP that produce clinically relevant effects by P2Y\textsubscript{12} antagonists are unclear, the present clinical data indicate that greater degrees of inhibition of ADP-mediated platelet aggregation are associated with significant reduction in stent thrombosis and more effective prevention of ischemic events.\textsuperscript{21}

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compared to high-dose clopidogrel.


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